ENZYME IMMOBILIZATION ON WOOLEN CLOTH

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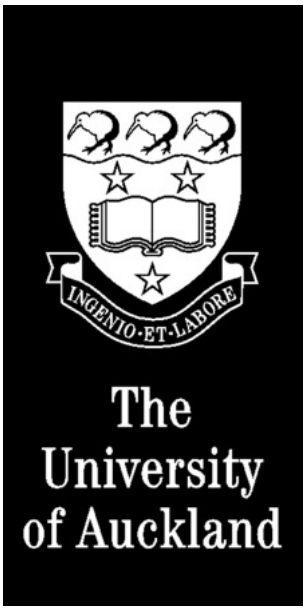
A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Engineering



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Abstract

The objective of this research was to determine the feasibility and the limitation of using woollen cloth as a support matrix for enzyme immobilization. Lipase from pseudomonas fluorescens was selected as the model enzyme. Covalent binding protocol (CVB) with a polyethyleneimine (PEI) spacer and glutaraldehyde (GA) cross-linker was used as benchmark protocol.

It was found that lipases could be effectively immobilized onto woollen cloth by the benchmark CVB protocol. The immobilized activity was independent of the GA concentration and soaking time in the GA solution, but dependent on the GA solution pH (optimal at pH 9). The treatment time in lipase solution was found to be the rate-determining step in the immobilization: the immobilized activity increased steadily within 5 to 10 hours of soaking in lipase solution, after that, the increase plateaued to a maximum of 4 μmol pNPP.min-1. (g cloth)$^{-1}$.

The immobilized lipase was reasonably stable: when the immobilized cloth was stored in 0.05 M Tris buffer (pH 8.5) for more than 80 days in a refrigerator, more than 80% of the lipase activity remained. The optimum pH for both free and immobilized lipase is approximately the same, which may indicate that microenvironment for the free and immobilised lipase was not considerably different. The Michaelis Menten parameters for the free and immobilized lipase were determined: K_M and v_{max} for the free and immobilized lipase were 2.40, 3.16 mM and 153.76, 5.16 μmol pNPP.min^{-1} (mg lipase)$^{-1}$ respectively. The lipase-immobilized cloth exhibited excellent oily stain removal ability: after being stained with olive oil and stored for one day in air at room temperature, the oily stain could be easily removed by 0.05 M pH 8.5 Tris buffer without any detergent addition. This enhanced cleaning was stable also, after cloth was stored in air for almost one month, similar cleaning performance was still observed.

Despite the successfulness in the benchmark CVB method, it was found to be a challenge to further improve the immobilised activity by variation of the CVB protocol and using different cross-linkers, although the ESEM images and measurement of protein load showed there was potential to do so. This was probably due to the fact that there are a number of reactive groups on wool surface which are not very reactive with the cross-linker used, thereby reducing the extent of surface activation, resulting in limited immobilized activity.

To further reduce the lipase deactivation during immobilization, immobilization of the lipase via assistance of another enzyme was tried in order to eliminate the use of GA. Thus, lipase was

entrapped inside a casein gel formed on woollen cloth in the presence of Transglutaminase (mTGase). Lipases were successfully immobilized by this protocol. Unavoidable lipase leakage and high mass transfer resistance from the casein gel however made this protocol not very promising compared to the CVB benchmark protocol.

The benchmark CVB immobilization protocol was also applied to galactosidase. The overall performance of the immobilized galactosidase was poor compared to the lipase however. This may indicate that the CVB immobilization method used is not immediately transferable to other enzymes and that modifications are required to do so. Further investigation is therefore needed.

Overall, the results from this project have shown that it is feasible to immobilize enzymes onto woollen cloth, although surface treatment of the wool to produce more binding groups would improve the surface coverage.

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List of Abbreviations

AU	Absorbance unit
BCA kit	Bicinchoninic acid protein assay kit
CVB	immobilization via covalent binding method
CRL	immobilization via cross-linking method
DCCA	Dichlorocyanuric acid
DI water	Deioned water
GA	Glutaraldehyde
K_M	Parameter of Michaelis-Menten kinetic model
M-M kinetics	Michaelis-Menten kinetics
mTGase	Microbial Transglutaminase
PEI	polyethyleneimine
PFL	Lipase from *pseudomonas fluorescens*
pNPP	p-nitrophenol palmitate
pNP	p-nitrophenol
pNPA	p-nitrophenyl acetate
[S]	The concentration of the substrate
Tris buffer	Trizma buffer
UV spectra	ultraviolet and visible adsorption spectra
v	Reaction rate
v_{max}	Maximum reaction velocity in Michaelis-Menten kinetic model